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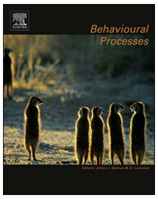

Brain size does not impact shoaling dynamics in unfamiliar groups of guppies (*Poecilia reticulata*)

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ABSTRACT

Collective movement is achieved when individuals adopt local rules to interact with their neighbours. How the brain processes information about neighbours' positions and movements may affect how individuals interact in groups. As brain size can determine such information processing it should impact collective animal movement. Here we investigate whether brain size affects the structure and organisation of newly forming fish shoals by quantifying the collective movement of guppies (*Poecilia reticulata*) from large- and small-brained selection lines, with known differences in learning and memory. We used automated tracking software to determine shoaling behaviour of single-sex groups of eight or two fish and found no evidence that brain size affected the speed, group size, or spatial and directional organisation of fish shoals. Our results suggest that brain size does not play an important role in how fish interact with each other in these types of moving groups of unfamiliar individuals. Based on these results, we propose that shoal dynamics are likely to be governed by relatively basic cognitive processes that do not differ in these brain size selected lines of guppies.

1. Introduction

Coordinated motion is observed in a number of taxonomic groups, including flocking birds, marching insects, and schooling fish (Parrish et al., 2002; Couzin and Krause 2003; Herbert-Read 2016). Collective motion is achieved when individuals adopt simple rules to interact with their neighbours (Couzin et al., 2002; Sumpter et al., 2012). These rules can include repulsion from neighbours that are too close, attraction to neighbours further away, and alignment with near neighbours' headings. Such rules allow individuals to transfer information on detected threats (Shimoyama et al., 1996; Procaccini et al., 2011; Herbert-Read et al., 2015; Rosenthal et al., 2015), track environmental gradients (Berdahl et al., 2013), and improve foraging success (Bazazi et al., 2012). Implementation of these rules requires individuals to monitor the position and trajectories of one or more neighbours, for which a range of cognitive processes are required. Here, we adhere to the definition of 'cognition' as comprising 'all mechanisms that invertebrates and vertebrates have for taking in information through the senses, retaining it, and using it to adjust behaviour to local conditions' (Shettleworth 2010). Note that this includes all aspects of cognition, from 'basic' perception of for instance salient moving objects to 'higher order' mental processes such as learning and decision-making. In the case of collective behaviour, stimuli generated from neighbours' movements need to first be detected and then integrated with an individual's internal state. These stimuli should then be used to inform movement decisions. For example, a pigeon flying in a flock collects visual information about the movements and positions of its neighbours. Retinal projections innervate several visual areas in the brain within the mesencephalon and diencephalon (Ehrlich and Mark 1984). Then, mostly within the caudolateral nidopallium, a proposed avian homologon to the mammalian dorsolateral prefrontal cortex (Emery and Clayton 2005), this visual information is integrated with additional information (such as internal state) to decide how to adjust flight speed and direction. Finally, the central pattern generators that control the pigeon's flight muscles (Grillner et al., 2008) elicit this decision. At present, understanding how animals move in groups has focussed on mapping individuals' movements in response to their neighbours' movements and positions (Herbert-Read et al., 2011; Katz et al., 2011; Gautrais et al., 2012), but this has not been linked to how the brain processes information during group movement. The next step in understanding collective motion, therefore, is to link behavioural responses to the underlying cognitive processes that govern these

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decisions.

Many comparative analyses support that brain anatomy is associated with behavioural complexity (Iwaniuk and Nelson 2001; Lefebvre et al., 2004; Sol et al., 2005). Recently, a number of studies have taken the field further by providing experimental data showing that brain anatomy affect cognitive abilities (Striedter 2005; Kotrschal et al., 2013b, 2014a, 2015; Benson-Amram et al., 2016); this is especially so when comparing animals within species (McDaniel 2005; Kotrschal et al., 2013b, 2014a), but can also be found when comparing animals within families (Benson-Amram et al., 2016) and even across orders (MacLean et al., 2014). Due to the vast differences in brain organisation, i.e. neuron size and number (Yopak et al., 2010; Herculano-Houzel 2017) across classes, larger-scale taxonomic comparisons are often not productive (Chittka and Niven 2009). Bumble bees, for example can perform amazing cognitive tasks with only very little cognitive substrate (Loukola et al., 2017). In the case of collective animal movement, properties of an individual's brain may play a central role in how it integrates information about the movements and positions of neighbours, and hence how it moves within groups. Cognitive aspects relevant to group behaviour may be found on several levels of brain organisation. For instance, more visually oriented animals generally have larger optic lobes (e.g. in birds, (Cobb 1964) and in fish, (Kotrschal and Palzenberger 1992), and differences in spatial orientation skills correspond to hippocampus size. This is the case in bird species that cache and retrieve food (Clayton et al., 2007) and in London taxi drivers (Maguire et al., 2000). Based on the reasoning that brain anatomy seems intricately linked with cognitive abilities and that group movement is governed by cognitive processes, we hypothesize that brain anatomy should impact collective animal behaviour.

Our aim was to determine if a key aspect of brain anatomy, relative brain size, influences how individuals move and interact in groups. We did so by comparing the group structure and movements of guppies that had been selectively bred for large or small relative brain size (Kotrschal et al., 2013b, 2014a). Animals from the large-brained lines have outperformed those from the small-brained lines in several tests of learning and memory (Kotrschal et al., 2013b, 2014a), but they show no difference in colour vision (Corral-Lopez et al., 2017a) or visual acuity (Corral-Lopez et al., 2017b). Moreover, the brains of these selection lines are proportionally similar in 11 main brain regions (Kotrschal et al., 2017b). This means that the absolute sizes of all regions are larger in large- compared to small-brained individuals. The guppy is a facultative shoaling species that typically initiates shoaling as a function of perceived threat (Magurran and Seghers 1994), with more aggregated shoals forming in stressful situations such as novel environments or the presence of predators (Dugatkin and Godin 1992). We made use of this shoaling tendency in novel environments and observed unfamiliar groups of two and eight male and female guppies selected for large and small brains fish in open arenas. Specifically, we tested for the effects of brain size selection on key aspects of swimming behaviour, such as speed, alignment, cohesiveness, and burst-and-glide characteristics; in the groups of eight we also examined the propensity to form subgroups and characteristics of these subgroups. These measures are fundamentally linked to the interaction rules individuals use when on the move (Perna et al., 2014), and should therefore reveal whether brain size influences collective motion in newly forming groups.

If the differences in cognitive abilities, created by brain size selection, indeed impact collective motion we predict that newly formed groups of large-brained individuals form 'better' shoals than such groups of small-brained individuals. This may be evident in faster group formation over time and/or overall better group coordination, which should lead to higher cohesiveness scores in large- compared to small-brained animals. Such 'better' shoals are typically characteristic of fishes evolving in predator-rich habitats and interpreted as anti-predator adaptations (Herbert-Read et al., 2017). Also, it has recently been suggested that the burst-and-glide motion, which can be found in many fish species (Weihs 1974), is the product of sequential information processing, in which fish decide about the direction and intensity of every burst based on the information collected during the glides (Herbert-Read et al., 2017). If this is the case, better information processing in large- compared to small-brained animals should alter the characteristics of this swimming pattern.

Considering that female guppies shoal more readily than male guppies (Croft et al., 2004) and previous experiments on these selection lines often have revealed female specific brain-size dependent differences (Kotrschal et al., 2015; van der Bijl et al., 2015; Kotrschal et al., 2016), we tested both sexes independently.

2. Material and methods

2.1. Directional selection on brain weight

We examined the relationship between brain size and shoaling dynamics in laboratory lines of Trinidadian guppies that were artificially selected for large or small brain size relative to body size (Kotrschal et al., 2012, 2013a). These selection lines were generated using a standard bidirectional artificial selection design that consisted of two replicated treatments (three up-selected lines and three down-selected lines). See Kotrschal et al. (2013a) for full details about the selection experiment. The selection lines differed in relative brain size by 9% in the F2 (Kotrschal et al., 2013a) and up to 14% in the F3 generation (Kotrschal et al., 2014a), and body size does not differ between the lines (Kotrschal et al., 2013a, 2014b). All fish were removed from their parental tanks after birth, separated by sex at the first onset of sexual maturation and then kept in single-sex groups with a maximum density of 12 (females) or 18 (males) individuals in 7 l tanks containing 2 cm of gravel and biological filters. We allowed for visual contact between the tanks. The laboratory was maintained at 26 °C with a 12:12 light:dark schedule. Fish were fed a diet of flake food and freshly hatched brine shrimp six days per week. We used 576 fully-grown and mature F3 male and female guppies for our assays, balanced over the three replicates, the two brain size selection regimes and both sexes (approx. 48 groups per treatment).

It is noteworthy that the population of guppies used for the brain-size selection experiment was only recently (4 years before the selection started) collected from the field and has since been kept in large semi-natural tanks. Inadvertent selection for 'tamer', more stress-resistant animals, and hence decreased shoaling propensity is therefore unlikely. This is corroborated by the fact that their behaviour (Corral-Lopez et al., 2017a,b) is very similar to wild-type guppies.

2.2. Arena assays

To determine shoal dynamics and quantify the coordination of newly formed groups of large- and small-brained animals, we conducted open field assays of groups. As the cognitive challenges of group coordination may depend on group size, we chose group sizes of two (smallest possible group) and eight (largest size the software can handle without compromising tracking efficiency). No fish was used twice, and each trial was composed of fish unfamiliar to each other and to the arena. We used four round, white arenas with 55 cm diameter and 3 cm water depth. The pair or group was first confined in the centre of the arenas for two minutes in an opaque white 15 cm PVC cylinder. After this acclimation period, the videos were started and the cylinders were lifted. We filmed each group for 10 min at 25 frames per second and tracked the individual movement of the fish using IDTracker (Pérez-Escudero et al., 2014). To minimize observer bias, blinded methods were used when all behavioural data were recorded and analysed. In total we performed 197 trials (33 female groups of eight, 36 male groups of eight, 66 female pairs, 62 male pairs).

To quantify shoaling dynamics we used the fine-grained tracking data to compute an exhaustive range of measures related to